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Brief Definitive Report

IDENTITY OF Leu-19 (CD56) LEUKOCYTE DIFFERENTIATION ANTIGEN AND NEURAL CELL ADHESION MOLECULE

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Cell surface receptors that are involved in cellular adhesion have been identified on several cell types in hematopoietic, muscle, and neural tissues (1, 2). This group of molecules includes fibronectin receptors, vitronectin receptors, platelet adhesion molecules, the leukocyte-associated CD11/CD18 and VLA glycoprotein complexes, and the neural cell adhesion molecule (N-CAM) glycoprotein (1, 2). These structures have been implicated in embryonic development, cell migration, and cellular adhesion, and often are highly conserved during evolution. The N-CAM antigen initially was identified in chicken neural tissue (1, 2). Chicken N-CAM is encoded by a single gene; however, numerous isoforms can be generated by differential RNA splicing and post-translational modifications, including the addition of *N*-linked carbohydrates and polysialic acid (2). Homologous N-CAM glycoproteins have been identified in human neural tissues (3). Recently, N-CAM cDNA isolated from human muscle tissue have been cloned and sequenced (4). Several isoforms of N-CAM exist, including modifications that permit attachment to the cell membrane by either a transmembrane polypeptide or phosphatidylinositol glycan (5).

The CD56 differentiation antigen, recognized by mAb anti-Leu-19 and NKH-1, is a ~175–185-kD glycoprotein that is expressed on essentially all human NK cells and on a subset of T lymphocytes and IL-2-activated thymocytes that mediate MHC-unrestricted cytotoxicity (6, 7). However, expression of Leu-19/NKH1 is not restricted to cytotoxic cells, since this antigen is present on certain $CD4^+$ Th cell clones, some myeloid leukemias, and the KG1a immature hematopoietic leukemia cell line (8). Herein, we report that Leu-19 also is expressed on neural tissues and is identical to N-CAM.

Materials and Methods

Cells. KG1a.5 is a cloned subline of the KG1a immature hematopoietic cell line that was selected for high levels of Leu-19 antigen on the cell surface (6). The SK-N-SH neuroblastoma cell line was generously provided by Ms. Roxanne Duan and Dr. Wolfgang Sadee (University of California, San Francisco). NK cells and Leu-19^+ T lymphocytes were isolated from peripheral blood and cultured in growth medium containing rIL-2, as described (6, 8).

Antibodies. Anti-Leu-19 mAb was produced as described (6). Rabbit antiserum against human N-CAM was generously provided by Dr. Frank Walsh (Institute of Neurology, London).

Immunoprecipitation, Deglycosylation, and Electrophoresis. Radioiodination, immunoprecipitation, and electrophoresis were performed as described (9). Immunoprecipitates were treated with 1 U/ml neuraminidase (from *C. perfringens*; Sigma Chemical Co., St. Louis, MO) in

Tris-maleate buffer (20 mM Tris-maleate, 10 mM D-galactono-γ-lactone, 1 mM calcium acetate, 0.5% NP-40, 0.02 mg/ml trypsin inhibitor, 1 mM PMSF, pH 6.0) for 4 h at 37°C. Digestion with Endo F was performed as recommended by New England Nuclear (Boston, MA). Peptide mapping was performed as described by Cleveland et al. (10), using protease type XVII from *Staphylococcus aureus* strain V8 (Sigma Chemical Co.).

Results and Discussion

N-CAM is a membrane glycoprotein expressed on neural and muscle tissues that is involved in homotypic adhesive interactions. Recently, we have observed that Leu-19 (CD56) is present on human neural tissues and neural-derived cell lines. In spinal cord, Leu-19 was strongly expressed throughout the gray and white matter. Cell surface staining was particularly prominent on the radiating glial septae of the white matter. In brain, Leu-19 was widely expressed throughout the molecular and granular layers of the cerebellum, as well as the cortex. These findings were consistent with the distribution of N-CAM on neural tissues and suggested that Leu-19 may be similar to N-CAM.

The presence of Leu-19 on neural tissues prompted us to examine whether it is related to N-CAM. KG1a.5, an immature hematopoietic cell line, and SK-N-SH, a neuroblastoma cell line, were labeled with ^{125}I and detergent solubilized. Antigens were immunoprecipitated using anti-Leu-19 mAb and rabbit anti-N-CAM serum. Samples were treated with neuraminidase and analyzed by SDS-PAGE. Anti-Leu-19 and anti-N-CAM immunoprecipitated a predominant band of ~145 kD from both the hematopoietic and neuroblastoma cells (Fig. 1 *A*). Mobility of Leu-19 was essentially identical when analyzed using both reducing (Fig. 1) and nonreducing conditions (not shown). Sequential immunoprecipitation confirmed that anti-Leu-19 and anti-N-CAM reacted with the same molecule (Fig. 1 *B*). Moreover, peptide mapping using *S. aureus* V8 protease indicated that the Leu-19 glycoproteins expressed on the hematopoietic and neuroblastoma cells were indistinguishable (Fig. 2). These results clearly demonstrate that anti-Leu-19 mAb reacts with the N-CAM molecule, and that the structure expressed on hematopoietic cells is similar to the form present on a neural cell line. Northern blot analysis revealed N-CAM transcripts in both KG1a.5 and NK cell lines (S. Cwirla and L. Lanier, unpublished observation).

Although N-CAM is encoded by a single gene, several isoforms can be generated by alternative splicing and differential polyadenylation (3, 5). Polypeptides of 180, 140, and 120 kD have been isolated from neural tissue, and 155-, 145-, and 125-kD proteins are present in muscle tissue (1, 2). The smaller forms (120 and 125 kD) are anchored to the cell membrane via a phosphatidylinositol glycan linkage, whereas the larger forms possess a transmembrane peptide segment (5). Additional diversity in N-CAM structure can be conferred by glycosylation, phosphorylation, sulphation, and sialyation (1, 2).

In hematopoietic tissues, Leu-19 is expressed on several cell types, including NK cells, T lymphocytes, and some myeloid leukemias (6–8). Since N-CAM can be expressed as several structural isoforms, further studies were conducted to compare the biochemical properties of Leu-19 expressed on hematopoietic cell types. Leu-19 immunoprecipitates prepared from ^{125}I-labeled lysates of NK cells, Leu 19$^+$ T cells, and KG1a.5 were treated with Endo F to remove *N*-linked carbohydrates and analyzed by SDS-PAGE. Deglycosylated Leu-19 expressed by NK cells and Leu-19$^+$ T

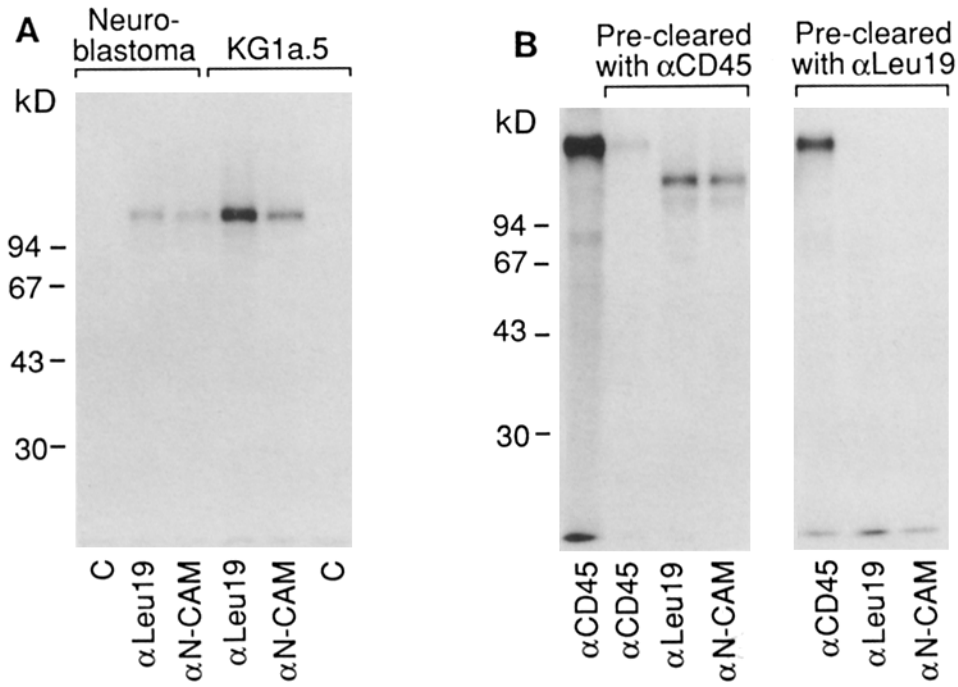

FIGURE 1. Immunoprecipitation of Leu-19 and N-CAM. (*A*) SK-N-SH neuroblastoma and KG1a.5 cells were labeled with ^{125}I and detergent solubilized. Lysates were immunoprecipitated with control Ig, anti-N-CAM, and anti-Leu-19. (*B*) ^{125}I KG1a.5 lysates were substantially depleted of Leu-19 or CD45 by immunoprecipitation with anti-Leu-19 and anti-CD45, respectively. After depletion, lysates were immunoprecipitated with anti-Leu-19, anti-CD45, and rabbit anti-N-CAM. Immunoprecipitates were treated with neuraminidase and analyzed by SDS-PAGE (reducing conditions) using 10% acrylamide gels. As reported previously (6), Leu-19 immunoprecipitates not treated with neuraminidase migrated as diffuse bands of ~175–185 kD (not shown).

lymphocytes is a 137-kD protein, similar to the polypeptide expressed on KG1a.5 (Fig. 3). Note that before deglycosylation, the mobility of Leu-19 isolated from normal T and NK cells was similar to the mobility of Leu-19 on KG1a.5, indicating that the extent of sialyation is similar in these cell types.

Further biochemical analysis of Leu-19 expressed on hematopoietic cells indicated that the glycoprotein was resistant to cleavage by Endo H and *O*-glycanase, enzymes that remove high mannose and *O*-linked carbohydrates, respectively (not shown). Therefore, it appears that the carbohydrates present on Leu-19 on hematopoietic cells are exclusively complex *N*-linked oligosaccharides with abundant sialyation. Leu-19 on hematopoietic cells was resistant to removal by phosphatidylinositol phospholipase C (PIPLC) (not shown). Given the size of the polypeptide (140 kD) and the inability to cleave Leu-19 using PIPLC, the molecule is likely anchored to the membrane via a transmembrane peptide.

An unusual structural feature of N-CAM is the abundance of sialic acid on this molecule (3). The extent of sialyation changes during embryonic development and can influence N-CAM binding function (11). We have shown that the epitope recog-

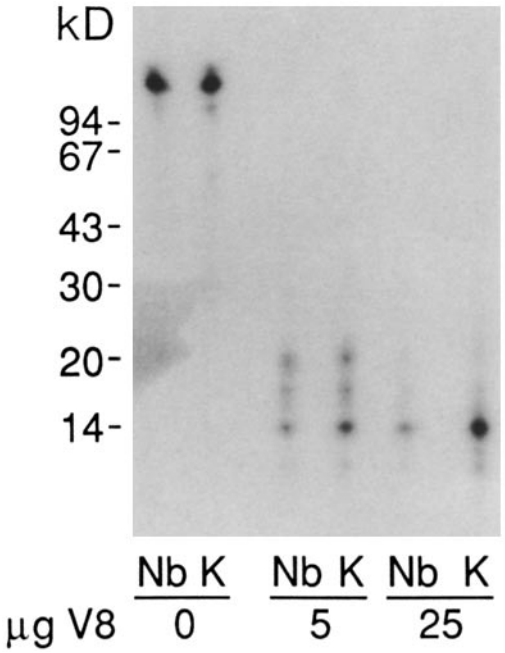

FIGURE 2. Peptide mapping of Leu-19. Leu-19 bands were cut from the SK-N-SH neuroblastoma (Nb) and KG1a.5 (K) lanes shown in Fig. 1, digested for 1 h with 0, 5, and 25 μg of *S. aureus* V8 protease, and analyzed by SDS-PAGE (reducing conditions) using a 15% acrylamide gel.

nized by anti-Leu-19 mAb is not dependent on sialic acid, since treatment of viable cells with neuraminidase did not diminish the binding of anti-Leu-19 as determined by flow cytometry (not shown). In fact, neuraminidase treatment increased binding of anti-Leu-19, indicating that Leu-19 epitopes are partially masked by sialic acid. We have observed that the amount of Leu-19 increases on NK cells after activation with IL-2 (unpublished observation), and that subsets of NK cells can be identified

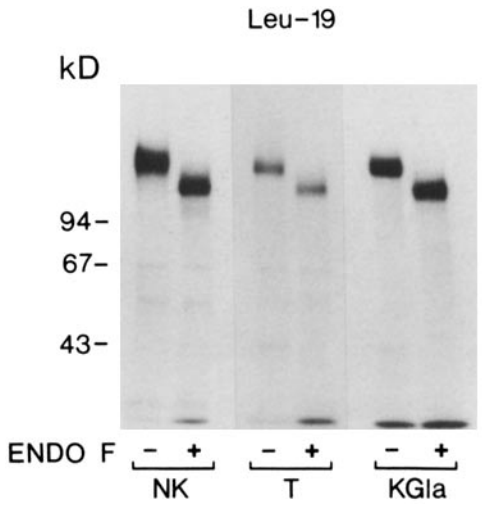

FIGURE 3. Immunoprecipitation of Leu-19 from NK cells, T cells, and KG1a.5. KG1a.5 cells, $CD3^-$, Leu-19^+ NK cells, and $CD3^+$, Leu-19^+ T cells were labeled with ^{125}I and detergent solubilized. Lysates were immunoprecipitated with anti-Leu-19. Leu-19 glycoproteins were deglycosylated with Endo F and analyzed by SDS-PAGE (reducing conditions) using 7% acrylamide gels.

based on relative density of Leu-19 expression (6). It will be interesting to determine whether this increase in Leu-19 is a result of de novo protein synthesis or alteration in sialyation. As in neural cells, sialyation may affect the function of Leu-19 on leukocytes.

In normal lymphoid tissues, Leu-19 is expressed predominantly on NK cells and a subset of T lymphocytes that mediate MHC-unrestricted cytotoxicity (6–8). Although this preferential distribution suggests that Leu-19 may be important in cytotoxicity, the function of Leu-19 on these cells has not been determined. Anti-Leu-19 mAb does not inhibit cytotoxic activity of NK or T cells against several tumor cell targets and does not affect IL-2-induced cellular proliferation (6–8). However, negative results from antibody blocking studies are inconclusive. Given the role of N-CAM in neural cell adhesion, it is conceivable that Leu-19 also may be involved in lymphocyte adhesion. Further studies will be necessary to examine this possibility.

Summary

Neural cell adhesion molecule (N-CAM) is a membrane glycoprotein expressed on neural and muscle tissues that is involved in homotypic adhesive interactions. We have demonstrated that N-CAM also is expressed on hematopoietic cells, and is recognized by the anti-Leu-19 mAb. Leu-19 is preferentially expressed on NK cells and T lymphocytes that mediate MHC-unrestricted cytotoxicity, but is also present on some myeloid leukemia cell lines. On NK cells, T cells, the KG1a.5 hematopoietic cell line, and a neuroblastoma cell line, Leu-19 is a ~140-kD polypeptide with *N*-linked carbohydrates and abundant sialic acid residues. Sequential immunoprecipitation and peptide mapping demonstrated that the Leu-19 and N-CAM molecules expressed on leukocyte and neuroblastoma cell lines are similar structures. These findings suggest that the Leu-19 antigen on leukocytes may be involved in cell adhesion, analogous to the function of N-CAM on neural cells.

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